Docosahexaenoic Acid and Childhood Blood Pressure

By
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Abstract

Background: Long chain polyunsaturated fatty acids (LCPUFAs) have been shown to reduce blood pressure (BP) while they are being consumed; however, there is also evidence from observational studies that exposure to more LCPUFA early in development can program lower BP in childhood. This relationship has yet to be studied in a randomized trial conducted during fetal life.

Objective: We tested the hypothesis that 600 mg/d of the omega-3 LCPUFA docosahexaenoic acid (DHA) compared to placebo in pregnancy can reduce offspring BP at 7 and 8 years of age.

Methods: This secondary data analysis examines 129 offspring from women who consumed capsules (placebo or 600 mg DHA) from <20 weeks of gestation to birth. BP was measured in triplicate at 7 and 8 years of age. The statistical analysis was intent-to-treat with adjustment for covariates associated with BP.

Results: We did not find an effect of early DHA exposure on BP, however, child weight status (BMI percentile < or $\geq 85^{th}$ percentile) and gestational days smoked were positively associated with BP at 7 and 8 years of age.

Conclusion: Improving maternal and therefore fetal DHA status through maternal DHA supplementation during pregnancy does not appear to protect against higher BP at 7 and 8 years of age.

Acknowledgements

I would like to thank my thesis advisor Dr. Susan Carlson for her help with completing this project. Her vast knowledge of DHA and pregnancy, enthusiasm for the subject, and guidance along the way has made this project possible. I would like to thank Elizabeth Kerling for her help with building my database and for always being available for questions and guidance. I would also like to thank Dr. Holly Hull for feedback on the project and for her help with the statistical analysis. This project would not have been possible without the help and support of these people along with the love and support from my family. Thank you.

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Chapter 1: Introduction

Long chain polyunsaturated fatty acids (LCPUFAs) have been shown to reduce blood pressure (BP) in adults (1).There is also some evidence from a randomized trial that exposure to LCPUFA during the first 4 months of life can program lower BP in childhood (2) however, the effect of fetal LCPUFA exposure on child BP has not been studied in a randomized trial. A randomized trial in Denmark looked at the effect of supplementation of docosahexaenoic acid (DHA) during the first 4 months of lactation and found higher BP in boys but not in girls at 7 years (3) and 13 years (4). Two more studies in Denmark studied dietary DHA intake at 8-11 years and 17 years and found higher amounts of DHA were associated with higher BP in children at 8-11 years (5) and 17 years (6).

In contrast, Vidakovic et al. (7) associated maternal DHA status in pregnancy with lower childhood BP at 6 years, and Forsyth et al. (2) found lower BP in children at 6 years who were provided a formula with DHA and arachidonic acid for the first 4 months of infancy. Studies by Ryter et al. (8), De Jong et al. (9), and Ayer et al. (10) found no relationship between DHA supplementation during the second trimester, the first 2 months of life, or over the first 5 years of life, respectively, and BP at 20 years, 9 years, and 8 years. Although multiple studies have been conducted on DHA and childhood BP, a consensus has yet to be reached.

No study to date has followed BP in children whose mothers were randomly assigned to DHA supplementation during pregnancy, thus exposing them to different amounts of DHA in the fetal environment. However, early reports from our own cohort suggest lower BP from 4 to 6 years in overweight/obese children whose mothers received 600 mg/d of DHA for the second half of pregnancy compared to overweight/obese children whose mothers received a placebo (under review). This and some of the observational studies suggest that there are long-term

programming effects of improving DHA exposure early in development. To examine, perhaps even longer term effects, we chose to measure BP in the children who had been studied from 4 to 6 years when they were 7 and 8 years of age.

Research Questions

Primary: Does supplementing with 600 mg/d DHA compared to a placebo during pregnancy result in lower BP at 7 and 8 years of age among the offspring?

Secondary: What factors predict increased BP at 7and 8 years of age?

Chapter 2: Review of Literature

Introduction

Heart disease is the number one cause of death worldwide (11). High blood pressure (BP) is a contributing factor to heart disease, and while it currently affects adults at a greater rate than children, hypertension rates in children are rising (12). Additionally, hypertension tracks from childhood into adulthood which is why early screening and intervention is important (12). While identifying and treating hypertension is important, finding ways to avoid hypertension altogether is a vital research mission in of itself.

Long chain polyunsaturated fatty acids (LCPUFAs) have been shown to decrease BP in adults (5), and researchers have begun to investigate whether the same BP-lowering effects present in adults are found in children. Data are mixed on the outcomes of early life postnatal DHA supplementation (2-4, 9, 10, 13). One observational study suggests prenatal exposure is related to lower BP in childhood (7), however, none of the current studies have examined random assignment to DHA exposure in developing fetuses and child BP. We have found lower SBP and DBP in children between 4 and 6 years of age who became overweight/obese if their mothers received DHA during pregnancy compared to overweight/obese children whose mothers received a placebo (14).

In summary, current research is inconclusive, and more randomized controlled trials conducted during fetal life are needed to show the effect of intrauterine LCPUFA exposure on childhood BP. We found an effect of early DHA exposure on later childhood BP when children were 4 to 6 years of age. The purpose of this study was to examine if supplementation with LCPUFAs continues to show a relationship to childhood BP in these same children when they are 7 and 8 years of age.

Hypertension Background

High blood pressure prevalence in children

National hypertension prevalence in children is about 3.5% (15). However, the rate is likely higher because hypertension is underdiagnosed in this population (16). According to Muntner et al, BP levels in children have increased in the past decade and mirror increases in obesity rates (17). When only looking at data of obese adolescents, the combined hypertension and pre-hypertension rates are about 30% (18). Although obese individuals are more susceptible to hypertension, children of all sizes can be affected, which is why screening is so important.

Hypertension diagnosis in children

Current recommendation is to check BP starting at age three (18). Early screening allows physicians to predict susceptibility to hypertension in the future (18). Diagnosis is based on set BP tables that have percentiles based on height, sex, and age for children ages 1 through 17 (18). BP ranges for children 1-13 years is shown in **Table 1.** Diagnosis of hypertension should be made after the confirmation of BP $\geq 95^{th}$ percentile at three different visits (19).

Table 1. Blood pressure ranges for children 1-13 years

Normal blood pressure	<90%ile
Elevated blood pressure	≥90%ile to <95%ile or 120/80 to <95%ile (whichever is lower)
Stage 1 hypertension	≥95%ile to <95%ile, plus 12mm Hg, or 130/80 to 139/89 (whichever is lower)
Stage 2 hypertension	≥95%ile , plus 12mm Hg, or ≥140/90mm Hg (whichever is lower)

Primary and secondary hypertension

Primary hypertension does not have a known origin, while secondary hypertension is caused by an underlying disease (16). Common causes of secondary hypertension include renal disease, heart disease, endocrine disease, family history, low birth weight, and being overweight (16). Other cardiovascular risk factors such as insulin resistance and dyslipidemia are often seen in adolescents with hypertension (20).

Health implications of high blood pressure in adults

While there are many causes of hypertension, it is important to get BP levels within healthy ranges to avoid negative health consequences. Although heart disease is thought to mainly affect adults, it could begin as early as infancy with hypertension and follow into adulthood, triggering many other problems (12). Coronary heart disease, stroke, and chronic kidney disease can all be associated with hypertension (21). Polyunsaturated fatty acids (PUFAs) could play a role in childhood hypertension.

Polyunsaturated fatty acid background

Essential fatty acids

Essential fatty acids are those that are essential to body function, but cannot be synthesized by the body, and therefore must be consumed from a food source or supplement

(22). The essential fatty acids include the omega-6 fatty acid, linoleic acid (LA), and its derivative arachidonic acid, and the omega-3 fatty acid, alpha-linolenic acid (ALA), and its derivatives eicosapentaenoic acid (EPA) and DHA (22). LCPUFAs that have been associated with BP are EPA and DHA.

LCPUFAs and dietary sources

While EPA and DHA can be converted from the shorter chained PUFA, ALA, this process is inefficient, meaning our bodies need to acquire direct sources of EPA and DHA (23). ALA is found in many food sources such as vegetable oils and walnuts; EPA and DHA are mainly found in fatty fish like salmon, tuna, mackerel, anchovy, and sardines (24). DHA is also an important component of human milk, but the levels vary based on genetics and maternal intake (25). As infant formulas try to simulate human milk as closely as possible, LCPUFAs are frequently added to formula (9).

LCPUFAs role in fetal and infant development

LCPUFAs are essential nutrients during fetal and infant development. EPA and DHA are especially important in fetal brain and retina development (26). DHA is considered more important than EPA as it is found in high concentrations in neural membranes and plays a major role in the function of the cell membranes (26). In contrast, EPA and EPA metabolites have many functions, but they are not found in high concentrations in cell membranes. Maternal intake of these essential fatty acids is directly related to the amount found in the fetus or infant (23). According to the U.S. Department of Health and Human Services, pregnant and breastfeeding mothers should consume 8-12 ounces of fish per week (23). If this guideline cannot be met, it is recommended to consume a daily supplement containing a minimum of 2.6 g omega-3 fatty acids with 100-300 mg of that being DHA (22).

LCPUFA role in blood pressure

In addition to playing a role in fetal and infant development, these LCPUFAs are thought to impact BP as well. LCPUFAs have been shown to lower BP in adults, but the effect in children is uncertain (5). LCPUFAs act as a hypotensive agent in adults by controlling sodium and water excretion, inhibiting the vasoconstrictor actions of thromboxane, interfering with vasopressor hormone responses, and acting as an anti-inflammatory agent (7). While these mechanisms of action are seen in adults, responses to LCPUFAs in children are less studied. In addition, there is the suggestion that early DHA exposure may program lower BP in early childhood and possibly even longer through some effect on early development that is not yet known.

Evidence that fish oil increases blood pressure

In the Danish study conducted by Asserhoj et al.(3) and Lauritzen et al. (2016) (4), participants were assigned to either a fish oil supplement group (containing 1.5g/d LCPUFAs) or an olive oil group for the first 4 months of lactation. Asserhoj et al. (3) examined 98 children while Lauritzen et al. (2016) (4) examined 103 Danish children. In the other Danish studies conducted by Damsgaard et al. (5) and Lauritzen et al. (2012) (6), dietary intake of the children was assessed by a 7-day recorded food record the week before BP was measured. The studies included 73 and 109 Danish children, respectively (5, 6). The children's BP was measured at 7 years (3), 8-11 years (5), 13 years (4), or 17 years of age (6).

Asserhoj et al. (3) found that BP did not differ in females, while males in the fish oil group had 6 mm Hg higher DBP and mean BP than compared with the males in the olive oil group. Damsgaard et al. (5) and Lauritzen et al. (2016) (4) found similar results in that LCPUFA

intake was associated with high BP in males only. Lauritzen et al. (2012) (6) found these same results in both males and females.

In a study analyzing 229 children living in the Netherlands, conducted by Seggers et al. (27), arachidonic acid and DHA levels were measured at birth from the umbilical cord. The children's BP was measured later at 9 years of age. It was found that greater arachidonic acid levels and a greater arachidonic acid to DHA ratio was associated with a lower DBP at 9 years of age. This is opposite of the findings expected in adults as arachidonic acid is considered adipogenic (27).

While these studies all found similar results, they still had limitations. The Lauritzen et al. (2012) (6) study and the Damsgaard et al. (5) study relied on food logs in order to determine LCPUFA intake. This method could be flawed as amount of food eaten can be inaccurately recorded or may not be an accurate representation of the children's actual intake (5, 6). Although blood samples were taken to analyze the fatty acid content, it is unknown how much LCPUFAs they were actually exposed to (5, 6). It is also unknown if supplementation during the first 4 months of life is a long enough intervention to exhibit the desired effects or if supplementation needs to occur even earlier in life (3, 4). While these studies all showed increases in BP, other studies have shown a decrease in BP with LCPUFA intake.

Evidence that fish oil decreases blood pressure

In the study examining 4,455 children conducted in the Netherlands (7), no LCPUFA supplementation was provided but the maternal LCPUFA levels were measured during the second trimester. In the study conducted by Forsyth et al. (2), which analyzed 235 infants from European centers located in Italy and United Kingdom, the infants were fed either a LCPUFAs

supplemented formula or an un-supplemented formula for the first 4 months of life. BP of both groups was measured at 6 years of age (2, 7).

Vidakovic et al. (7) found that greater maternal omega-3 PUFA and specifically DHA levels were related to lower offspring SBP at age 6, while DBP remained unchanged. However, the differences were only -0.28 and -0.29 mm Hg per SD increase of total maternal omega-3 PUFAs and DHA weight % respectively (7). Another study from the same cohort analyzed the data using a principal component analysis model which allowed them to better analyze the patterns of fatty acids present in the mothers during pregnancy and they did not find an effect of maternal plasma fatty acid patterns on childhood BP (13).

Breastfeeding appeared to have a beneficial relationship with offspring BP. The breastfed control children had similar BP levels to children born to mothers with greater omega-3 levels (7). Vidakovic et al. (7) also observed that a greater omega-6 to omega-3 ratio was related to a greater SBP. Forsyth et al. (2) found similar results in which the LCPUFAs supplemented infants had a lower mean and DBP at 6 years of age. The researchers examined the design and discovered the following possible study limitations.

Vidakovic et al. (7) examined maternal LCPUFA status during the second trimester. This may not be an accurate representation of fetal LCPUFA status, as this can vary based on the amount that is actually transferred to the fetus (7). While the infants in the Forsyth et al. study received supplemented formula for the first 4 months of life, the diet after this period was not evaluated, leaving room for the children to consume unknown amounts of LCPUFAs (2). And again, supplementing during the first 4 months of life may not be a long enough or early enough exposure time. Other studies have found LCPUFAs to have no effect on childhood BP.

Evidence that fish oil has no relationship with blood pressure

In the study conducted by Ayer et al. (10), 616 Australian families were supplied with canola-based oils and spreads to use in cooking and eating. They were also instructed to give their child a daily tuna oil capsule (135mg DHA/capsule). The control groups were given sunola oil to use in their diet (10). These diet interventions were implemented from the time they started eating solids until 5 years of age (10). The study conducted by Ryter et al. (8) measured maternal LCPUFAs levels from 443 Danish mothers during the second trimester. The study conducted in the Netherlands by Pluymen et al. (28) compared BP at 5 years of age of 2,468 newborns fed infant formula supplemented with LCPUFA and those who consumed formula without LCPUFAs. In the final study conducted by De Jong et al. (9), 474 infants from the Netherlands received formula supplemented with LCPUFAs (0.35% by wt DHA) from birth until 2 months of age. These children were compared to infants given standard formula and those who were breastfed (9).

The children's BP was measured at 8 years (10), 20 years (8), 5 years (28) and 9 years (9). In all of these studies, no differences were found between those who had greater LCPUFA intake during pregnancy (8), infancy (9, 28), or early childhood (10) when compared to those who had less.

The researchers examined the design and discovered the following possible study limitations. Ayer et al. (10) suggested a reason for the findings was an insufficient amount of LCPUFAs. While the canola-based and tuna oils positively altered the omega-3 to omega-6 ratio, the omega-3 levels remained below those found in areas where fish intake is high (10). De Jong et al. (9) noted that the short supplementation period of 2 months could be a limiting factor as longer supplementations periods had a greater effect on BP.

Conclusion

As multiple studies have resulted in various conclusions, the answer still remains as to how LCPUFAs affect BP in children. While LCPUFAs play an important role in the development of infants and children, their effect on childhood BP is still unknown. Flaws in some of these experiments include limited supplementation time, supplementation during the incorrect periods of time, and an insignificant amount of LCPUFA. Also none of these studies were conducted in the United States. Further research on LCPUFAs and BP is needed to clarify the effects LCPUFAs have on adolescents' BP. Research conducted in which DHA is supplemented during pregnancy is needed to assess DHA's impact during fetal development.

Chapter 3: Methods

Overview of Parent Trial

This study is designed to test the effect of 600 mg/d of DHA supplementation during pregnancy on childhood BP at 7 and 8 years of age. A total of 350 women from Kansas City metropolitan area between the ages of 16 and 35.99 and between 8 to 20 weeks of gestation were recruited from January 2006 and November 2009 to participate in a study on the effect of DHA supplementation on gestation duration and early infant cognition (29). After the conclusion of the primary and secondary studies, the women were asked to continue in a tertiary study to measure the children's body composition and BPs at 7 and 8 years of age.

Setting

The original cohort for the KU DHA Outcomes Study (KUDOS) trial was recruited from the Kansas City metropolitan area. They were recruited from January 2006 to November 2009. The study was conducted from 2006 to 2011 (29).

Ethics

Both the research protocol and informed consent process were in compliance with the Declaration of Helsinki, and were approved by the Institutional Review Boards/Human Subjects Committee at the University of Kansas Medical Center, the University of Missouri-Kansas City; and St Luke's Hospital. The original trial was registered at clinicaltrials.gov as NCT00266825 (29).

Subjects

Three hundred fifty healthy, English-speaking pregnant women between the ages of 16 to 35.99 and 8 to 20 weeks of gestation who planned on delivering in the Kansas City metropolitan area were recruited to participate. Exclusion criteria for the original trial included BMI ≥40, expecting multiple infants, preexisting diabetes mellitus or SBP ≥140 mm Hg at enrollment, or had any serious health condition likely to affect development and growth of the offspring. These could include cancer, lupus, hepatitis, HIV/AIDS, or a diagnosed alcohol or chemical dependency (29).

Randomization

After participating in the informed consent process, eligible women were randomized into either a corn/soy oil placebo group (0 mg DHA) or a marine algae-oil source (600 mg DHA). Both supplements were provided by DSM Nutritional Products, Columbia, MD. Regardless of group women were instructed to consume 3 capsules/day until birth. The women were encouraged to consume the 3 capsules/day, but if this was not possible, the women were told to consume as many capsules as they could up to 3 capsules/day. The participants were blinded to the randomization until the children were 6 years of age and had completed early cognitive and visual acuity development testing. The study team will remain blinded until completion of the study (29).

Capsules

A month's supply of capsules was mailed to the participants and the participants were asked to mail the unused capsules back to the investigational pharmacy in a prepaid envelope.

The pharmacy then counted and recorded the unused capsules and destroyed the remaining capsules. This count was used to determine capsule intake for the participants. A minor financial incentive was provided for mailing back the used capsules (29).

Blood Analysis

A blood sample was collected from participants at enrollment and the morning after delivery. Venous cord blood was obtained at the time of birth. This blood was analyzed for fatty acid content by way of gas chromatography. Blood samples were collected by venipuncture into 5-mL sodium–EDTA tubes (Vacutainer; Becton-Dickinson) and placed on ice immediately. Plasma and red blood cells (RBCs) were separated by centrifugation (3000 3 g, 10 min; 48C), frozen, and stored under nitrogen at 2808C until analyzed (29). Lipids were isolated according to a modification of Folch et al (30), and RBC lipids were fractionated (31) by thin-layer chromatography. RBC phospholipids were transmethylated with boron trifluoride- methanol (32), and the resulting fatty acid methyl esters were separated by using a Varian 3900 gas chromatograph with an SP-2560 capillary column (100 m; Sigma Aldrich) as previously reported (33) and a Star 6.41 Chromatography Workstation for peak integration and analysis. Injector and detector temperatures were programmed at 2608C. The column temperature program for the 41-min column run was as follows: 5 min at 140^{o} C, 4^{o} C increase/min to 240^{o} C, and held at 240^{o} C for 11 min. Individual peaks were identified by comparison with qualitative standards (PUFA 1 and PUFA 2; Sigma Aldrich), and a weighed standard mixture (Supelco 37 Component FAME mix; Sigma Aldrich) was used to adjust fatty acids for area/weight to calculate a final percentage weight of total fatty acids. RBC-phospholipid-DHA is reported as a percentage of total fatty acids by weight (29).

KUDOS Body Composition Study

Of the 350 women enrolled in the parent KUDOS trial, 172 were assigned to the placebo group and 178 were assigned to DHA. At the completion of the 6 year project, a total of 166 participants completed their visit, with 81 children remaining in the placebo group and 85 in the DHA group. These families were asked to continue in an ancillary study, known as the KUDOS Body Composition (KUDOS Body Comp) follow-up from ages 7 to 9 years old. The primary outcome of KUDOS Body Comp was to determine the influence of prenatal DHA on child fat and fat free mass during late childhood. Food intake, physical activity, body composition, height, weight, heart rate, and BP were measured.

After informed consent of the parent and assent of the child, families were asked to come to the study site once a year near the child's birthday for one visit which lasted about 1 hour. During which time the parent(s) (with the assistance of child if needed) filled out questionnaires regarding child exposure to foods with DHA, physical activity, and parenting styles. A 24-hour recall was also conducted. Six site (sub-scapular, triceps, biceps, abdominal, hip and thigh) skin fold measurements were measured at least in duplicate according to standard laboratory practice as well as child BP (process expanded below), and the BodPod body composition measurement according to industry standards.

Blood Pressure Measurements

SBP and DBP measurements were recorded three times by trained staff using an automated sphygmomanometer. BP was measured after the children had been lying down for 2-3 minutes while their sagittal abdominal diameter was measured. The BP measurement was taken using an appropriate cuff size on the left arm and the cuff was kept at the level of the heart.

Proper cuff size was chosen by ensuring the index line on the cuff was between the indicated marks on the cuff. A smaller or larger cuff size was chosen if these index lines did not fall between the designated markers. The cuff was wrapped around the arm, with the arrow marked artery aligning with the patient's brachial artery. Heart rate was also measured three times in a row with the same equipment as the BP. Average SBP and DBP measurements were calculated using the last two measures, and the coefficient of variance (CV) determined for each average. If the CV was ≥0.095, the two measures of the three taken that were closest were averaged instead.

Materials

The data collected included sex, race, height, weight, heart rate, and BP of the children and the corresponding amount of DHA given during pregnancy, maternal pre-pregnancy body mass index (BMI), gestational days smoked, and days breast fed. The equipment utilized included a sphygmomanometer, stadiometer, and scale. Child BMI percentile was calculated using EZ-BMI calculator software version 2013. In accordance with standard practice, the software used the child's age, sex, date of birth, date of measurement, height, and weight to determine the child's BMI percentile.

Statistical Analysis

All statistical analyses were conducted with SPSS 25 (IBM). The dependent variables for this study were SBP and DBP (mm Hg) at 7 and 8 years. The following potential variables were examined with respect to child blood pressure: breastfeeding, gestational weight gain, child and maternal weight status, gestational days smoked, and child race and sex. A correlation matrix was built using the Pearson's correlation to identify any relationship between SBP or DBP at 7

and 8 years and child weight status, gestational days smoked, and pre-pregnancy weight status. Analysis of covariance (ANCOVA) was used to identify any effect of DHA assignment (placebo or 600mg DHA) for SBP and DBP at 7 and 8 years. The ANCOVA was adjusted for child weight status, gestational days smoked, pre-pregnancy weight status, child sex, and child race because there are previously reported relationships with these variables.

Chapter 4: Results

The primary purpose of the study is to determine if DHA supplementation during pregnancy influences BP at 7 and 8 years of age. Our hypothesis is that DHA supplementation results in lower BP in childhood. A secondary purpose of this study is to find predictors of increased childhood BP. Our hypothesis is that a predictor of increased childhood BP is child weight status. A total of 129 children participated in the cohort at 7 and 8 years. Of these, 63 were randomly assigned to placebo and 66 to DHA (**Table 2).** We have BP measurements for 117 children at 7 years of age and 106 children at 8 years of age.

Table 2. Characteristics of participants

Characteristic* (Total n=129)	Placebo (n=63)	DHA (n=66)
Maternal pre-pregnancy weight status (%)		
Underweight/normal weight	60	43
Overweight/obese	40	57
Smoked during pregnancy (%)	37	35
Average days smoked among those who smoked	155	144
Breast fed (%)	87	79
Average days breast fed among those who breastfed	226	253
7 year child weight status (%)		
$<85^{th}$ percentile	80	69
$\geq 85^{th}$ percentile	20	31
8 year child weight status (%)		
$<85^{th}$ percentile	78	71
$\geq 85^{th}$ percentile	22	29
Child race (%)		
African American	37	29
Not African American	63	71
Child ethnicity (%)		
Hispanic	6	6
Not Hispanic	94	94
Child sex (%)		
Males	54	41
Females	46	59
DHA change cluster (%)		
Increase	12	66
Flat	88	34
Average change in DHA from enrollment to delivery (% red blood cell-phospholipid-DHA by weight)	+0.290765	+3.274828
Average blood pressure (Systolic/Diastolic)		
7 year	103/62	102/62
8 year	104/64	104/63
Child fish and DHA supplement intake (average times/week)		
7 year	1.3	1.1
8 year	1.3	0.8

*Some participants have missing data for some of the above characteristics

Analysis of covariance

Table 3 shows the ANCOVA results for 7 year SBP and DBP. There is no significant difference between placebo and DHA group for 7 year SBP or DBP (P = 0.625 and P = 0.431, respectively). **Figures 1 and 2** show the means for 7 year SBP and DBP for placebo and DHA groups. The means are adjusted for 7 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

Table 3. Analysis of covariance for 7 year blood pressure

	Average Systolic Blood Pressure (mm Hg)	Standard Deviation	95% Confidence Interval		P value[b]
			Lower Bound	Upper Bound	
7 Year Systolic Blood Pressure					
Placebo	102.2[a]	11.3	100.0	104.4	0.625
DHA	101.4[a]	10.7	99.3	103.5	0.625
7 Year Diastolic Blood Pressure					
Placebo	62.3[a]	7.8	60.8	63.9	0.431
DHA	61.5[a]	7.4	60.1	62.9	0.431

[a]Means appearing in the model are adjusted for 7 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.
[b]P value <0.05 is significant

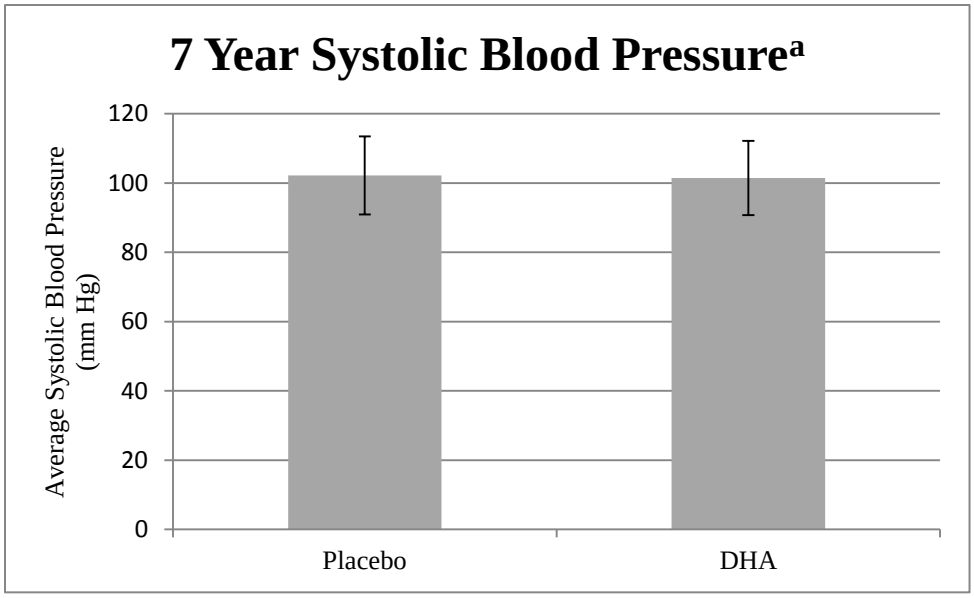

Figure 1. 7 year systolic blood pressure
[a]Means appearing in the model are adjusted for 7 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

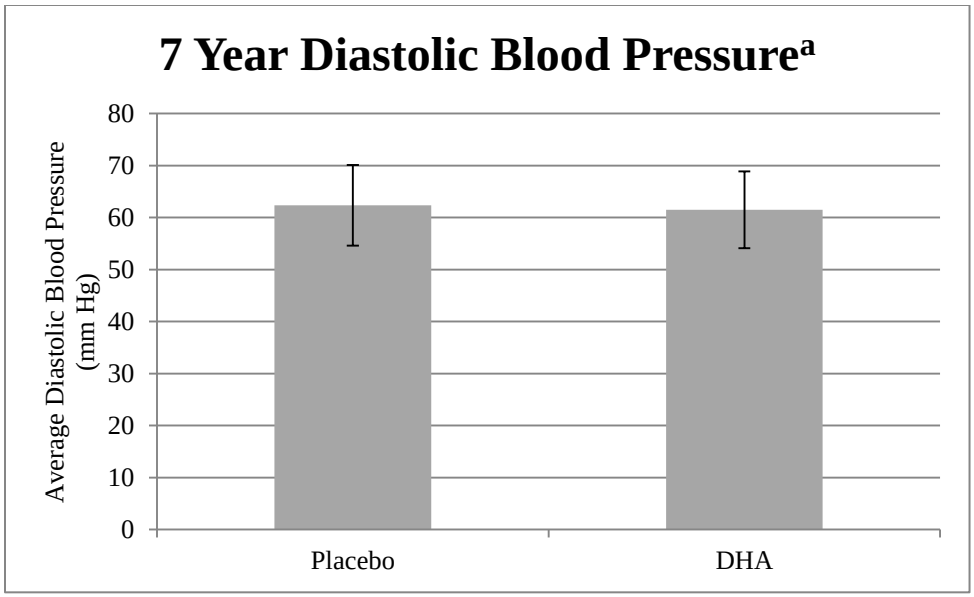

Figure 2. 7 year diastolic blood pressure
[a]Means appearing in the model are adjusted for 7 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

Analysis of covariance

Table 4 shows the ANCOVA analysis for 8 year SBP and DBP. There is no significant difference between placebo and DHA group for 8 year SBP or DBP (P = 0.973 and P = 0.510, respectively). **Figures 3 and 4** show the means for 8 year SBP and DBP for placebo and DHA groups. The means are adjusted for 8 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

Table 4. Analysis of covariance for 8 year blood pressure

	Average Systolic Blood Pressure (mm Hg)	Standard Deviation	95% Confidence Interval		P value[b]
			Lower Bound	Upper Bound	
8 Year Systolic Blood Pressure					
Placebo	104.0[a]	9.9	102.0	106.0	0.973
DHA	104.0[a]	10.0	101.9	106.0	0.973
8 Year Diastolic Blood Pressure					
Placebo	63.6[a]	6.4	62.3	64.9	0.510
DHA	63.0[a]	6.5	61.7	64.3	0.510

[a]Means appearing in the model are adjusted for 8 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.
[b]P value <0.05 is significant

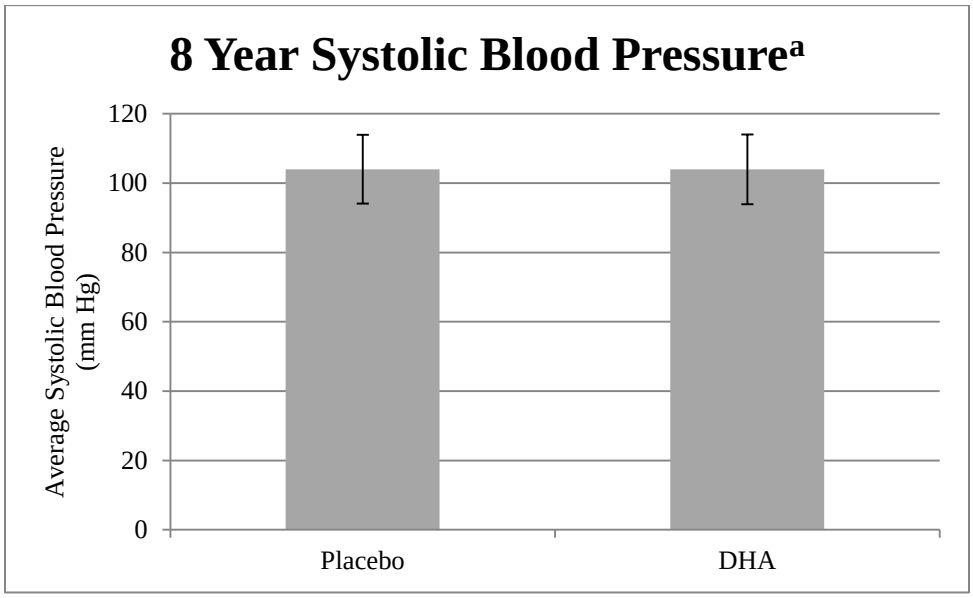

Figure 3. 8 year diastolic blood pressure
[a]Means appearing in the model are adjusted for 8 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

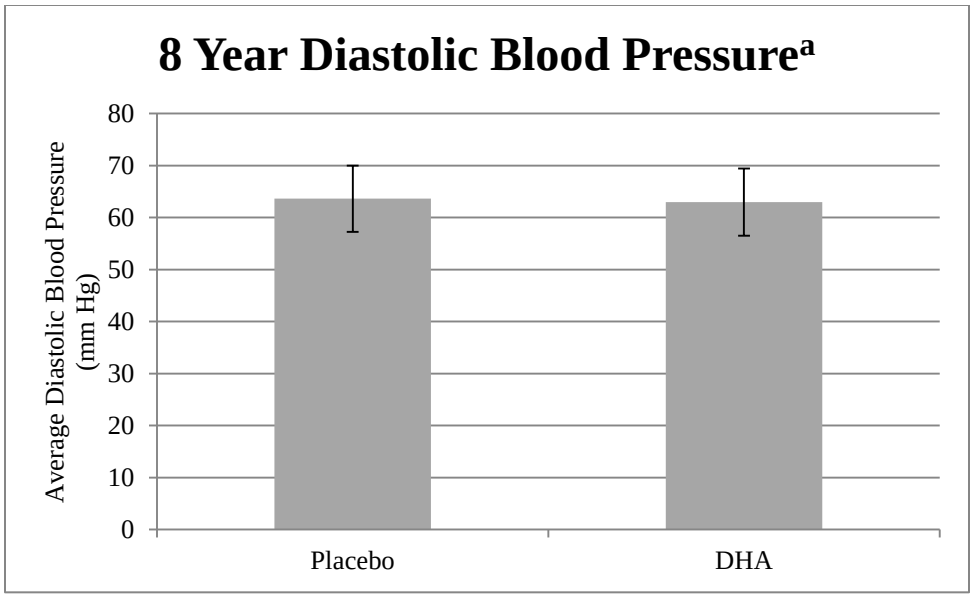

Figure 4. 8 year diastolic blood pressure
[a]Means appearing in the model are adjusted for 8 year weight status, gestational days smoked, pre-pregnancy weight status, child race, and child sex.

Correlations

Table 4 shows the correlation for 7 and 8 year SBP and DBP. For 7 year SBP, 7 year weight status (P = <0.001) and gestational days smoked (P = <0.001) are positively associated with 7 year SBP. For 7 year DBP, 7 year weight status (P = 0.015) is positively associated with 7 year DBP. For 8 year SBP, 8 year weight status (P = <0.001) and gestational days smoked (P = 0.005) are positively associated with 8 year SBP. For 8 year DBP, none of the values are significant. At both 7 and 8 years, child weight status was positively correlated with gestational days smoked (P = 0.004 and P = 0.002, respectively).

Table 4. Correlation table for 7 and 8 year blood pressure

	R Value	P value[a]
7 year SBP[b]		
7 year weight status	0.494	**<0.001**
Gestational Days Smoked	0.336	**<0.001**
Pre-pregnancy weight status	-0.027	0.771
7 year DBP[b]		
7 year weight status	0.224	**0.015**
Gestational Days Smoked	0.085	0.365
Pre-pregnancy weight status	-0.010	0.913
8 year SBP[c]		
8 year weight status	0.501	**<0.001**
Gestational Days Smoked	0.271	**0.005**
Pre-pregnancy weight status	0.125	0.202
8 year DBP[c]		
8 year weight status	0.164	0.094
Gestational Days Smoked	0.143	0.144
Pre-pregnancy weight status	-0.102	0.300

[a]P value <0.05 is significant; values in bold are significant
[b]n = 117
[c]n = 106

Chapter 5: Discussion

Does supplementing with 600 mg DHA compared to a placebo during pregnancy result in lower BP at 7 and 8 years of age? We did not find evidence to support it does. For the secondary research question, what are possible predictors of increased BP at 7and 8 years of age?, we find childhood weight status and gestational days smoked to be predictors of higher BP in 7 and 8 year old children.

Maternal DHA assignment is not shown to be significant at 7 or 8 years of age for either SBP or DBP. These results do not support our hypothesis that DHA has a protective effect on childhood BP. These results agree with previous studies conducted on LCPUFAs and BP (8-10, 28). However, other studies have found the LCPUFAs can decrease BP (2, 7) or that LCPUFAs can increase BP (3-6, 27). The studies showing a decrease in BP with higher LCPUFA levels examined the children's BPs at 6 years of age. Our previous study did show significantly lower BP at 4-6 years of age in overweight/obese children (14). This could suggest that as children become older the protective effect of early DHA exposure on childhood BP in overweight/obese children is overcome by the effect of continued overweight/obesity.

It is clear from the study that an overweight/obese weight status at both 7 and 8 years of age leads to a much higher SBP. The effect on DBP, however, is smaller or absent. Child weight status is shown to be significant at 7 years with SBP and DBP and at 8 years with SBP only. Children $\geq 85^{th}$ percentile had significantly higher SBP at 7 and 8 years of age and DBP at 7 years. Increased weight is a well-known risk factor for hypertension in adults, and this relationship is also seen in children (34-37).

Maternal pre-pregnancy weight status did not prove to be significant at 8 years of age with either SBP or DBP. A review by Ludwig-Walz et al. (38) reviewed 16 research articles

analyzing the relationship between maternal pre-pregnancy weight status and offspring's BP. This systematic review concluded that the evidence was suggestive, but limited. Maternal pre-pregnancy weight may be associated with offspring weight, which then causes high BP (38).

The dispersal of overweight/obese mothers is unevenly distributed as about 57% of the mothers in the DHA assignment group are overweight/obese, and 40% of the mothers in the placebo group are overweight/obese. This uneven distribution is also seen in the children's weight status at 7 and 8 years. At 7 years, about 20% of the children in the placebo group and 31% of the children in the DHA group are $\geq 85^{th}$ percentile and at 8 years, about 22% of the children in the placebo group and 29% of the children in the DHA group are $\geq 85^{th}$ percentile. This puts the children in the DHA group at somewhat of a disadvantage as obesity is related to higher BP.

Several studies have found a positive association between maternal smoking and childhood BP (39-42). Li et al. (43) found that maternal smoking was associated with childhood obesity which could lead to increased BP, but smoking itself was not associated with higher BP. Bergel et al. (44) did not find an association between smoking and childhood BP either. We find a positive relationship with 7 and 8 year SBP and gestational days smoked. We also find a positive correlation between gestational days smoked and childhood weight status at 7 and 8 years ($P = 0.004$ and $P = 0.002$, respectively; results not shown). More research is needed in this area to confirm the association between maternal smoking and childhood BP.

Other Research/Future Research

Childhood BP is a common area of study as hypertension is becoming more prevalent. A study by Lindberg et al. (45) supplemented children from 6 weeks to 6 months of age with iron or a placebo. The study found that the low birth weight children supplemented with iron had a

protective effect resulting in lower BP at 7 years of age. This and research like it open the door for other studies examining vitamins, minerals, or fatty acids to see what potential relationship they may have with childhood BP. More studies are needed to find other possible contributors to increased BP.

Implications of Findings

While DHA supplementation does not prove to be significant at 7 or 8 years of age, the findings that child weight status and gestational days smoked impact childhood BP gives us further reason to improve the rising obesity rates in the country. Other research, like the Lindberg et al. (45) study give us further reason to keep investigating different nutritional factors that could improve BP.

The main limitation of this study is that BP is not the primary outcome of the original study. Therefore, the methods used to measure BP may not be the most optimal. While the children were sitting and lying down prior to the measurements, some of the children were active between tasks or talking during the measurements, which could result in inaccurate readings. Although the children were instructed to remain still and quiet during the measurements, the children were not always compliant.

Possible limitations from the original cohort include that some prenatal vitamins that contain DHA were introduced to the market during the time of enrollment. Women who consumed less than 300 mg DHA from other sources were not excluded from this study. Twenty seven percent of the participants from the original cohort took additional DHA. Dietary DHA intake was not recorded from the mothers, but the blood analysis is a sensitive measure to DHA intake (29).

Conclusion

While DHA supplementation did not prove to have an effect on childhood BP at 7 and 8 years of age, we did find an impact of childhood weight status and gestational days smoked.

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